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STANDARD TEST FISH DEVELOPMENT

PART II

Chronic Toxicity of Guthion to the Fathead Minnow

(*Pimephales promelas* Refinesque)

by

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ABSTRACT

Three chronic bioassays of Guthion® were conducted with fathead minnows. All tests were begun with eggs and the longest lasted 20 days after termination of spawning, a total of 250 days. Parameters measured were survival, growth, fecundity, and growth and survival of second generation fry and eggs. The most sensitive criteria for effect of the toxicant was fecundity. The maximum acceptable toxicant concentration was between 0.33 and 0.51 µg/liter Guthion,® and the application factor was between .0017 and .0027 based on a soft water acute and a hard water chronic bioassay.

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Criteria	Chronic toxicity	06F
Fecundity	Bioassay survival	06S
Growth	Application factor	
Minnows	Maximum acceptable toxicant concentration	
Water quality		
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SECTION I

CONCLUSIONS

The results described in the following report permit certain conclusions concerning the toxicity of Guthion® to fathead minnows.

1. The maximum acceptable toxicant concentration for fathead minnows is between 0.33 and 0.51 µg/liter Guthion.®

2. The application factor comparing an acute bioassay in soft water and a chronic bioassay in hard water is between .00017 and .00027.

SECTION II

RECOMMENDATIONS

Guthion® concentrations in the environment should not exceed 0.33 µg/liter.

SECTION III

INTRODUCTION

Guthion® (azinphosmethyl) is an important and widely used insecticide on deciduous fruits and cotton as well as many other fruits and vegetables. Although acute toxicity data have been reported by many workers for a variety of fish species (Katz, 1961; Pickering, Henderson, and Lemke, 1962; Meyer, 1965; and Macek and McAllister, 1970), no information has been reported on the effect of exposures over a complete life cycle. Since data on chronic toxicity are important for establishment of water quality standards, fathead minnows were exposed to various concentrations of Guthion® over a complete life cycle, from egg through fry in the second generation, in order to determine a maximum acceptable toxicant concentration (MATC) (Mount and Stephan, 1967).

SECTION IV

MATERIALS AND METHODS

NUMBER OF EXPERIMENTS

Three experiments were conducted, subsequently referred to as tests I, II, and III. Tests I and II were preliminary and were terminated after 26 and 68 days, respectively, when it became apparent that the lowest concentration was causing significantly greater mortality than the control. Furthermore chemical analysis of small Guthion® concentrations had not been perfected, and residues on glassware and water sampling equipment made analyzed concentrations inaccurate. This problem was particularly true for test II where desired concentrations were low enough to be influenced. Therefore, information from the first two tests is only roughly quantitative but does indicate an area of possible effect. For test III all glassware was soaked in a strong sodium hydroxide solution (pH = 12^{+}) for at least two days prior to use and no residues were found in blank analyses.

APPARATUS

A proportional diluter (Mount and Brungs, 1967) that dispensed seven toxicant concentrations with a 60% dilution factor and one control was used for administration of the toxicant in a flow-through system. Technical grade Guthion® (93%) was added directly to a single mixing chamber by the injector described by DeFoe (1975). An air dispersion stone and baffles thoroughly mixed the Guthion® with incoming water before it was siphoned to the toxicant cells in the diluter. The test chambers for first generation young and adult fish were constructed of

glass and G.E. Silicone Seal, measured 50 x 25 x 20 cm, and contained 20 liters. With a flow rate of 250 ml/min, 90% replacement of water occurred in approximately 3-1/4 hr. Temperature was maintained at a constant 25 C by a hot water exchange system in the head tank where aeration also occurred. All test chambers in test III contained an air dispersion stone in order to maintain oxygen concentrations. A dense bacterial growth occurred in the two highest concentrations apparently using the Guthion® as a nutrient and the aeration was necessary to replace oxygen consumed by the bacterial respiration. Chambers were siphoned daily except when fry were present to keep this bacterial growth to a minimum. A complete analysis of hard water from a deep well is reported in Table 1. Those water quality characteristics which varied in individual test chambers are listed in Table 2.

Fry in the second generation were reared in glass chambers measuring 20 x 20 x 21 cm and containing 6.4 liters. For each concentration water flowed from the outlet of the adult test chamber into the first fry chamber, then from the outlet of that to a second fry chamber so that two groups of offspring from the same adults could be reared simultaneously. Eggs were hatched in a small jar with a nylon screen bottom. One jar in each adult test chamber was oscillated so that water flowed past the eggs without raising them from the screen.

All adult chambers were exposed to the direct illumination of a 40-watt cool white fluorescent tube, suspended 50 cm above the water surface. Second generation fry chambers received only indirect illumination from laboratory light. Tests were begun with a photoperiod equivalent to January 1 at Evansville, Indiana and adjustments were made every 15 or 16 days.

FISH

Fathead minnows originally obtained from the Environmental Research Laboratory at Duluth, Minnesota were reared in a laboratory culture system. All tests were started with eggs from five spawning pairs which

Table 1. ANALYSIS OF WELL WATER[a]

(milligrams/liter)

Item	Concentration
Total hardness as $CaCO_3$	220
Calcium as $CaCO_3$	140
Iron	0.02
Chloride	<1
Sulfate	<5
Sulfide	0.0
Fluoride	0.22
Total phosphates	0.03
Sodium	6
Potassium	2
Copper	0.0004
Manganese	0.0287
Zinc	0.0044
Cobalt, nickel	<0.0005
Cadmium, mercury	<0.0001
Ammonia, nitrogen	0.20
Organic nitrogen	0.20

[a]Water taken from well head before aeration and heating; pH 7.5.

Table 2. MEANS AND STANDARD DEVIATIONS (IN PARENTHESES) OF CONDITIONS IN ALL TEST CHAMBERS

Item	Test chamber 1	2	3	4	5	6	7	8
				Test I				
Guthion® (µg/l)	0	7.6(1.3)	11.7(1.9)	20.5(1.9)	34.5(3.2)	61.4(2.4)	96.7(6.6)	165.9(13.1)
Temp. (C)	24.8(.6)	25.0(.6)	24.9(.6)	25.0(.6)	25.0(.6)	24.9(.6)	25.1(.6)	25.1(.6)
pH (reading)	7.73(.13)	7.53(.11)	7.52(.10)	7.49(.09)	7.51(.11)	7.52(.16)	7.50(.09)	7.56(.17)
DO (mg/l)	6.60(.66)	5.95(.24)	5.55(.56)	5.03(.05)	5.20(.74)	4.48(1.28)	4.46(.61)	5.13(.51)
				Test II				
Guthion® (µg/l)	0	1.8(.9)	1.9(1.0)	2.8(1.1)	3.4(1.2)	4.8(.8)	6.5(.8)	15.0(2.2)
Temp. (C)	24.6(1.7)	24.6(1.8)	24.6(1.9)	24.4(1.9)	24.6(1.9)	25.4(.6)	25.3(.6)	25.4(.6)
pH (reading)	7.75(.09)	7.58(.11)	7.57(.10)	7.60(.09)	7.60(.09)	7.58(.08)	7.60(.09)	7.63(.10)
DO (mg/l)	6.28(.77)	5.54(.45)	5.55(.45)	5.58(.31)	5.36(.38)	5.22(.57)	5.17(.61)	4.90(1.07)
				Test III				
				Adult chambers				
Guthion® (µg/l)	.04(.03)[b/]	.10(.04)	.16(.05)	.24(.06)	.33(.08)	.51(.11)	.72(.15)	-
Temp. (C)	24.1(1.1)	24.4(1.2)	24.4(1.2)	24.4(1.2)	24.6(1.4)	24.5(1.4)	24.4(1.2)	-
pH (reading)	8.08(.11)	8.06(.11)	8.07(.11)	8.06(.11)	8.05(.11)	8.05(.11)	8.06(.12)	-
DO (mg/l)	6.96(.34)	6.52(.57)	6.49(.34)	6.27(.64)	5.79(.59)	5.77(.90)	5.61(.86)	-

Table 2 (continued). MEANS AND STANDARD DEVIATIONS (IN PARENTHESES) OF CONDITIONS IN ALL TEST CHAMBERS

Item	Test chamber 1	2	3	4	5	6	7	8
				Test III				
				Fry chamber 1				
Guthion® (µg/l)[c]	.04(.03)[b]	.10(.04)	.16(.05)	.24(.06)	.33(.08)	.51(.11)	.72(.15)	-
Temp. (C)	23.6(.8)	23.8(.5)	23.8(.4)	23.8(.5)	23.9(.4)	23.6(.6)	23.6(.4)	-
pH (reading)	8.22(.08)	8.17(.04)	8.15(.05)	8.16(.04)	8.12(.02)	8.15(.10)	8.14(.08)	-
DO (mg/l)	7.30(.41)	6.86(.22)	6.81(.30)	6.65(.33)	6.17(.41)	6.00(.76)	5.87(.60)	-
				Fry chamber 2				
Temp. (C)	23.0(.6)	23.0(.6)	22.9(.4)	23.0(.6)	23.1(.6)	23.2(1.0)	22.4(1.4)	-
pH (reading)	8.23(.08)	8.16(.05)	8.16(.05)	8.15(.05)	8.13(.07)	8.18(.08)	8.15(.08)	-
DO (mg/l)	7.46(.32)	6.96(.32)	7.09(.29)	6.99(.34)	6.45(.51)	6.49(.84)	6.06(.83)	-

[a] Concentration may be high by as much as 50% for the four lowest concentrations.
[b] Unknown source of contamination resulted in some Guthion® in control.
[c] Concentrations measured in adult chambers.

were distributed in a random stratified manner to egg chambers in each toxicant concentration and control. Initial numbers of eggs were 90, 70, and 100 in tests I, II, and III, respectively. Eggs were in the middle to late blastula stage when first exposed to the toxicant and more than 99% were fertile. Mortality of eggs and fry was recorded at 24-hr intervals in tests I and II. In test III eggs were checked at 24-hr intervals but mortality of young was recorded only after 22 days to avoid increased stress from handling. At that time two runts or malformed fish were removed from each chamber and fish were randomly thinned to 15 fish.

Ten-cm sections of transite pipe were split lengthwise to form a spawning substrate. After 90 days six of these sections were placed in each aquarium, three on each end with an open area in the center. As secondary sexual characteristics developed, males were removed at bi-weekly intervals to keep no more than five per chamber. Spawning substrates were checked at 1000 hours each day and if spawning occurred, eggs were counted and a random sample of 50 was placed in the egg basket for determination of survival through hatching unless the basket was already occupied. Two groups of fry from each concentration were reared for 50 days, otherwise all hatched fry were discarded.

Fry were fed ground Glencoe trout pellets and live brine shrimp twice a day for the first 30 days. Older fish were fed Oregon Moist frozen pellets twice a day and live brine shrimp once a day.

CHEMICAL ANALYSIS

Concentrations of Guthion® in each adult test chamber were usually analyzed twice per week for the first 5 months of test III and usually once per week thereafter. Samples from either or both fry chambers were analyzed periodically. Since the percentage difference between fry chambers and adult chambers analyzed at the same time ranged from -24% to +21% with a mean percentage difference of ±0%, the analyses from adult chambers were used.

A 1-liter sample of water from each test chamber was extracted with 10 ml benzene (pesticide grade) and the benzene was then evaporated to 0.5 to 2 ml, depending on the concentration. With each series of samples 1 liter of deionized water was spiked with 1 ml Guthion® in acetone and this sample was treated the same as all others to determine a recovery efficiency which averaged 99.2 ± 6.5%. Samples were analyzed on a Beckman GC 72-5 gas chromatograph with a 122-cm glass column packed with 50% DC 200 on 80/100 mesh Gas Chrom-Q and a nonradioactive electron capture detector. Standards were prepared from 93% technical grade Guthion® and were injected alternately with unknowns. Final Guthion® concentrations were corrected for the recovery efficiency and the technical grade product.

The pH was measured with a meter twice per week in adult chambers and once per week in fry chambers when they were occupied. Temperature was measured with a thermister three times per week in all chambers and dissolved oxygen was analyzed weekly by the azide modification of the Winkler analysis.

SECTION V

RESULTS

EGG AND FRY SURVIVAL

Survival of eggs was probably not directly affected by any concentration of Guthion® tested. In Test I percentage survival through hatching decreased from 90 to 29 to 17% as Guthion® concentration increased from 34.5 to 61.4 to 96.9 µg/liter but then survival increased to 76% as Guthion® concentration increased to 165.9 µg/liter (Table 3). This decreased survival was caused by eggs in these concentrations being enmeshed in a bacterial network that apparently used Guthion® as an energy source and grew in proportion to the concentration. Dead eggs in chambers with reduced survival were partly hatched as the fry seemed to become enmeshed in the bacteria and could not break free. Since the chorion of these eggs softened, fry may also have been weakened. Survival in the highest concentration was apparently greater because most eggs hatched 1 day sooner, when the bacteria were less dense and before the chorion had softened.

At 22 days there was decreased survival of fry in comparison to controls at 7.6 µg/liter in Test I, and at approximately 6.5 µg/liter in Test II (Table 3). By 57 days survival decreased at approximately 1.8 µg/liter Guthion® in Test II but did not decrease at 0.51 µg/liter in Test III. In Test III Guthion® caused no decrease in survival at any life history stage with the possible exception of the highest concentration where fry were lost when the chamber overflowed. The MATC in terms of survival was between 0.51 µg/liter in Test III and a maximum of 1.8 µg/liter

Table 3. PERCENTAGE SURVIVAL OF FATHEAD MINNOW EGGS AND YOUNG EXPOSED TO VARIOUS CONCENTRATIONS OF GUTHION®

Item	Test chamber 1	2	3	4	5	6	7	8
				Test I				
Guthion® (µg/l)	0	7.6	11.7	20.5	34.5	61.4	96.9	165.9
% hatched	99	84	97	95	90	29	17	76
% survival								
22 days	54	43	29	16	1	0	0	0
				Test II[a]				
Guthion® (µg/l)	0	1.8	1.9	2.8	3.4	4.8	6.5	15.0
% hatched	91	92	90	90	87	93	63	84
% survival								
22 days	57	48	57	43	48	48	37	35
57 days	54	42	34	5	3	0	0	0
98 days	52	35	21	0	0	0	0	0
				Test III				
Guthion® (µg/l)	.04[b]	.10	.16	.24	.33	.51	.72	-
% hatched	86	84	82	85	85	86	83	-
% survival								
22 days	61	80	74	82	76	67	[c]	-
from 22-60 days	100	100	93	100	100	93	88	-

[a] Concentration may be high by as much as 50% for the four lowest concentrations.

[b] Unknown source of contamination resulted in some Guthion in the control.

[c] Tank overflowed and fry were lost.

where increased mortality occurred in Test II.

GROWTH

In test II fish were weighed twice en masse during the experiment and at termination. Each time the control fish weighed more than those in the lowest concentration, 1.8 µg/liter (Table 4). In test III the first two weighings were of individual fish that were randomly thinned from the experiment. The third weighing was of fish that had remained in the experiment. Individual t-test comparisons indicated no significant difference between the control and any of the treatments ($p>.05$) at 22 days (Table 4). At 60 days the 0.10 and 0.24 µg/liter treatment levels weighed significantly more than the controls ($p<.02$) but since the 0.16 µg/liter treatment level was not different from the control, Guthion® was probably not the cause of the above differences. After 90 days there was no significant difference ($p>.05$) between the control and any treatment. Although dissolved oxygen concentrations were lower with increasing Guthion® concentrations, these oxygen levels did not seem to affect growth.

By 120 days males in some treatments started to defend territories and fish were not weighed to avoid any influence of handling on spawning behavior. At termination of the experiment after 250 days there was no consistent pattern of growth related to Guthion® concentration. Therefore, Guthion® affected growth of fathead minnows through 90 to 100 days at essentially the same concentrations that caused mortality, or between 0.72 and 1.8 µg/liter.

REPRODUCTION

Interpretation of fecundity data was complicated by the variation in sex ratios in test chambers. Although an attempt was made to maintain five males and seven females in each chamber, in some chambers that many males were not produced, in some immature males could not be identified, and in some individuals were lost to accidental death or escapement.

Table 4. MEAN WEIGHT OF FATHEAD MINNOWS AT VARIOUS TIME INTERVALS AFTER SPAWNING (NUMBER OF FISH WEIGHED IN PARENTHESES)

Item	Test chamber 1	2	3	4	5	6	7	8
				Test II				
Guthion® (µg/l)	0	1.8	1.9	2.8	3.4	4.8	6.5	15.0
Weight (mg)								
57 days	162(33)	111(29)	55(21)	72(3)	20(2)	-	-	-
85 days	320(33)	250(24)	230(14)	-	-	-	-	-
98 days	380(33)	350(24)	300(13)	-	-	-	-	-
				Test III				
Guthion® (µg/l)	0.4	0.10	0.16	0.24	0.33	0.51	0.72	
Weight (mg)								
22 days[a/]	24(8)	24(27)	28(25)	27(19)	23(21)	21(11)	-	
60 days[a/]	179(15)	242(15)	216(13)	231(15)	260(15)	165(13)	-	
90 days[b/]	580(15)	580(15)	640(15)	660(14)	600(15)	600(13)	720(15)	
250 days[c/] - male	2834(3)	3047(5)	3455(5)	3051(5)	2628(5)	2965(6)	3136(5)	
female	1434(9)	1594(7)	1458(5)	1353(5)	1558(5)	1360(6)	912(1)	

[a/] Fish randomly thinned from experiment.
[b/] Fish remaining in experiment.
[c/] Termination.

Spawning occurred after 121 days when the day length reached 12 hr and 45 min. Spawning ceased after 230 days when the day length was reduced to 12 hr. Intensive spawning occurred first at 0.33 µg/liter Guthion®, followed 12 to 14 days later by spawning at 0.24, 0.16 and 0.10 µg/liter. Intensive spawning by the control fish occurred 29 days later and 56 days later in 0.51 and 0.72 µg/liter Guthion® although at 0.51 µg/liter spawning occurred infrequently during the test. The number of spawnings and eggs were greatest in the control chamber (Table 5). This result may have been caused by the highest female to male sex ratio throughout the test. The more significant measurements in terms of Guthion® effect were the number of eggs per spawning and per female since these normalize the dissimilar sex ratios. On this basis Guthion® affected fecundity between 0.33 µg/liter which was similar to controls and 0.51 µg/liter where there was about a 50% reduction in eggs per spawning and per female (Table 5). The decreased oxygen concentrations with increasing Guthion® was apparently not responsible for the effect on fecundity since the oxygen concentration at 0.33 and 0.51 µg/liter Guthion® was similar.

Table 5. FECUNDITY OF FATHEAD MINNOWS EXPOSED TO VARIOUS CONCENTRATIONS OF GUTHION®

Guthion® µg/l	Number of spawnings	Number of eggs	Number of eggs per spawning	Number of eggs per female[a/]
0.04	88	16401	186	1691
0.10	54	8541	158	1220
0.16	57	9567	168	1611
0.24	49	7091	145	1239
0.33	52	8590	165	1718
0.51	21	1791	85	256
0.72	27	2408	89	782

[a/] Computed by intervals between thinnings.

SECOND GENERATION

Random samples of 50 eggs were taken from 12 spawnings from each test chamber except the second highest where samples from seven spawnings were taken. These eggs were hatched in the oscillating egg baskets. As with the first generation eggs, there was no effect of Guthion® at the tested concentrations (Table 6).

Table 6. EFFECT OF GUTHION® ON SURVIVAL AND GROWTH OF SECOND GENERATION EGGS AND FRY

Item	Guthion® concentration, µg/l						
	0.04	0.10	0.16	0.24	0.33	0.51	0.72
% survival - eggs	86.2	84.2	90.9	86.2	82.3	86.4	90.2
% survival - fry[a]							
1st group	79	97	100	97	89	-[b]	78
2nd group	69	89	88	89	100	--	100
Mean weight (mg)[a]							
1st group	94	130	94	126	115	78	128
2nd group	143	102	135	85	144	222	80

[a] After 50 days from hatching.

[b] Some fry lost due to overflow.

Survival or growth of fry was not consistently affected by the tested Guthion® concentrations (Table 6). Survival was poorest among controls. Considerable differences in growth occurred between test chambers but these differences were not related to Guthion® concentrations (Table 6).

SECTION VI

DISCUSSION

The lowest concentration of Guthion® that had an adverse effect on fathead minnows was 0.51 µg/liter where fecundity was much reduced (Table 5). An estimation of the maximum acceptable toxicant concentration based on this parameter is between 0.33 and 0.51 µg/liter Guthion®. Effects on survival and growth during early life history stages occurred at slightly higher concentrations (Tables 3 and 4). Although no effect occurred in test III at the highest concentration (0.72 µg/liter) growth and survival were reduced at the lowest concentration (1.8 µg/liter) in test II.

The 96-hr LC50 for 11-week-old fathead minnows tested in a relatively soft water was 1900 µg/liter (Part I of this report). An application factor based on this acutely toxic concentration and the MATC in the present study is between .00017 and .00027. Although this application factor is extremely small compared to factors found for many other toxicants (U.S. Environmental Protection Agency, 1973), it would probably be even smaller if the acute tests were conducted in hard water. Henderson, Pickering and Tarzwell (1960) found that the 96-hr LC50 of Guthion® to fathead minnows was approximately 25 to 40% less in soft water than in hard (20 mg/liter vs. 400 mg/liter total hardness). This differential in hardness was more extreme than the acute vs. chronic test in the present study, but if the application factor was based on an acute test conducted in hard water, the implication is that the factor would be even smaller.

SECTION VII

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SECTION VIII

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Adelman, I.R., L.L. Smith, Jr., and G.D. Siesennop. Chronic Toxicity of Guthion to the Fathead Minnow (*Pimephales promelas* Rafinesque). Bulletin of Environmental Contamination and Toxicology (submitted).

SECTION IX

GLOSSARY

Adult - A fish older than 4 months.

Application Factor - The ratio of the MATC to the 96-hr LC50.

Bioassay - A toxicity test; the estimation of the strength of a poison by its effect on a living organism.

Bioassay, acute - A toxicity test of short duration, usually less than 3 weeks.

Bioassay, chronic - A toxicity test of long duration, usually 1 month or longer.

Bioassay, flow-through - A toxicity test where the toxicant and diluent water are continuously replaced by fresh material.

Blastula - Early embryonic stage where cellular division has resulted in cells arranged as a hollow spherical body.

Chorion - The outer membraneous covering of a fish egg.

Fry - A fish younger than about 50 days.

LC50 - The concentration of poison that will kill 50% of the test organisms at a specified time.

Maximum Acceptable Toxicant Concentration (MATC) - A concentration of toxicant determined from a chronic bioassay and considered an estimation of the level at which no harm will occur in the natural environment. It is bracketed by the highest level of no effect and the lowest level of effect in the bioassay.